THE MORPHOLOGIC AND SEROLOGIC RELATIONSHIPS OF THE VARIOUS FUNGI CAUSING DERMATITIS VERRUCOSA (CHROMOBLASTOMYCOSIS)

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THE MORPHOLOGIC AND SEROLOGIC RELATIONSHIPS OF THE VARIOUS FUNGI CAUSING DERMATITIS VERRUCOSA (CHROMOBLASTOMYCOSIS)[1]

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The literature concerning the fungi pathogenic for man is confusing because of the constantly changing terminology and reclassification of the etiologic agents. Many of the changes in classification have been the result of studies of incomplete collections of fungi or studies based on descriptions rather than a working knowledge of the fungi. One of the most confusing and chaotic situations in this respect is that pertaining to the fungi causing dermatitis verrucosa (chromoblastomycosis). One of the organisms found in this disease, *Hormodendrum Pedrosoi* Brumpt 1922 (1), has been placed by various observers in five different genera, namely: *Acrotheca* (2), *Trichosporium* (3, 4), *Gomphinaria* (5), *Botrytoides* (6), and *Phialoconidiophora* (6). Such a situation is confusing not only to the medical man whose knowledge of the fungi is necessarily limited but also to the well trained mycologist. Obviously, for such a situation to exist, either the fungi reported or the genera proposed are imperfectly known.

In a previous report (7) we have shown that a single spore culture of the strain isolated from the North Carolina case developed structures which could be used as evidence for placing the fungus in at least three different genera. Serologic studies from the same case suggested a very close antigenic relationship between strains of fungi isolated from chromoblastomycosis in different

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parts of the world. With this as a basis, we have made comparative and serologic studies of seventeen strains of fungi isolated from cases of dermatitis verrucosa to make their identity more easily recognized. These studies have permitted the identification of four morphologically different fungi each of which may cause similar clinical lesions: *Phialophora verrucosa* Thaxter 1915 (8), *Hormodendrum Pedrosoi* Brumpt 1922 (1), *Hormodendrum Langeroni* Fonseca, Leão and Nogueira-Penido 1927 (9), *Hormodendrum compactum* Carrion 1935 (10).

MATERIALS AND METHODS

The 17 strains studied came from cases of chromoblastomycosis showing a wide geographical distribution including South America, North America, Puerto Rico, Costa Rica, Uruguay, Guatemala, and Algeria. Two of the 17 strains were found to be *Phialophora verrucosa*, thirteen *Hormodendrum Pedrosoi*, and one each *Hormodendrum compactum* and *Hormodendrum Langeroni*. The fungi studied and their sources are listed below:

1. *Phialophora verrucosa* Thaxter 1915

Phialophora verrucosa	204	Texas case*
Phialophora verrucosa	283	Uruguay

2. *Hormodendrum Pedrosoi* Brumpt 1922

Trichosporium Pedrosoi	38	South America
Trichosporium Pedrosoi	229	South America
Acrotheca Pedrosoi	268	South America
Hormodendrum Pedrosoi	274	South America
Hormodendrum Pedrosoi	279	South America
Hormodendrum Pedrosoi	280	South America
Hormodendrum Pedrosoi	69	Puerto Rico
Hormodendrum Pedrosoi	267	Puerto Rico
Hormodendrum Pedrosoi	275	Puerto Rico
Hormodendrum Pedrosoi	284	Puerto Rico
Hormodendrum Pedrosoi	269	North Carolina
Hormodendrum Pedrosoi	297	Guatemala
Hormodendrum algeriensis	281	Algeria

* *Phialophora verrucosa* 270 designated as from Medlar's Boston case in a previous report (7) was later found to be the A.T.C.C. strain from the Texas case (12).

3. *Hormodendrum compactum* Carrion 1935
   Hormodendrum compactum 277 Puerto Rico
4. *Hormodendrum Langeroni* Fonseca, Leao and Nogueira-Penido 1927
   Hormodendrum Langeroni 282 C.B.S. (Costa Rica)

The gross and microscopic characters of these fungi were studied on Sabouraud's glucose agar and corn meal agar both in petri dish and slide cultures.

The serologic studies were based on the sera of rabbits immunized with two typical strains of *Hormodendrum Pedrosoi* 269 and 281 and one strain each of *Hormodendrum compactum* 277, *Phialophora verrucosa* 283, and *Hormodendrum Langeroni* 282. The fungi were grown at room temperature for two weeks. The growth from three Sabouraud's slants was scraped off the medium with a large flattened loop. The material was then ground with a mortar and pestle for several minutes and suspended in 10 cc. saline and shaken with sterile glass beads for one hour in a shaking machine. One cubic centimeter of the suspension was injected intracutaneously into each of five areas of the shaven flank of one rabbit; the total material injected consisted of 5.0 cc. of a suspension containing a two-weeks growth of the fungus of approximately 1½ Sabouraud's slants. Three series of injections were given at weekly intervals and the serum was tested five days after the last inoculation. Complement fixation tests were then carried out to demonstrate antigenic relationships.

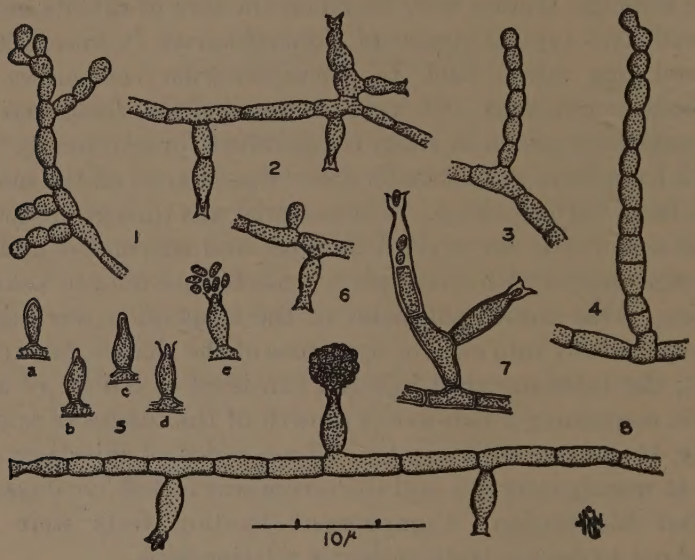
DESCRIPTION OF THE FOUR TYPES

1. *Phialophora verrucosa* Thaxter in Medlar, Mycologia **7**: 200–203, 1915; Journ. Med. Res. **32**: 507–523, 1925. Plate 2, figures 3, 4, 5 and text figure 1.

This fungus was first isolated from a tumor on the buttocks of an Italian in Boston (11). The lesion, however, was not comparable to those found later in South American cases. In 1933 a case reported from Texas (12) gave lesions similar to those reported from South America. This fungus has since been found in Uruguay (13).

In culture the North American strain differed markedly from

the Uruguay strain. On Sabouraud's agar (plate 2, fig. 3 and 5) it developed a heaped granular greenish brown growth covered with a short nap-like aerial mycelium. At the periphery in three weeks a grayish aerial mycelium developed. The cultures were slow growing and the heaped type of development cracked the agar. In contrast, the culture from Uruguay (plate 2, fig. 4) developed as a close matted greenish brown aerial growth with a slightly grayish border. This culture failed to heap and crack



TEXT FIG. 1. PHIALOPHORA VERRUCOSA

1, 3 and 4, moniliform hyphae developed in the agar; 2, 6, phialides developed singly, branched, or subtended by one cell; 5, development of the phialide and spores; 7, abnormal development of phialide; 8, characteristic conidiophores, phialides, and spore group.

the agar as did the other and looked very similar on Sabouraud's glucose agar, to *Hormodendrum Pedrosoi* 281 (plate 2, fig. 2).

Microscopically the two cultures of *Phialophora* were identical. Each produced conidia endogenously from flasked-shaped conidiophores (phialides). These phialides developed as lateral buds (text fig. 1, *6*) (text fig. 1, *5a*) and usually near a septum on the hyphae. When approximately 6 μ long, a small bud-like process was visible at the tip, (text fig. *5b*). As this bud developed the

wall of the phialide became thicker at the junction of the bud (text fig. 1, *5c*) and finally ruptured at the tip leaving a cup-like enlargement through which conidia could be pinched off from the growing point in the phialide (text fig. 1, *5d* and *e*). The conidia were held at the mouth of the cup in a compact mass. These spore balls were more easily seen in Van Tieghem cell preparations and slide cultures. In lacto-phenol mounts the conidia which had developed on phialides of the aerial hyphae were dispersed and the sphere like groups were not readily apparent. The conidia were thin-walled, egg-shaped cells 1.5 μ wide by 4 μ long. In the groups at the mouth of the phialides they appeared smaller. These conidia germinated readily and their development could be easily followed in Van Tieghem cells. The phialides were borne terminally as well as laterally on the aerial mycelium (text fig. 1, *8*). They were 3 to 4 μ in diameter and 4 to 7 μ long; occasionally longer phialides were seen. Sometimes these phialides were grouped or were subtended by a single cell (text fig. 1, *2*); or could be seen as elongated structures (text fig. 1, *7*). In the agar the vegetative mycelium produced lateral branches which became moniliform in character (text fig. 1, *1*, *3*, *4*). These moniliform hyphae were also found in the submatrical mycelium of cultures of *Hormodendrum*.

Phialophora verrucosa differs from the other forms by the production of conidia only from flask-shaped conidiophores; this is the only type of conidial formation known for this form. (Compare text fig. 1, *8*; plate 3, fig. 3, *9*, *13*).

Cultures studied:

Phialophora verrucosa 204, Texas case
Phialophora verrucosa 283, Uruguay

2. *Hormodendrum Pedrosoi* Brumpt, Precis de Parasitol. ed. 3, page 1105, 1922.

Synonyms:

Acrotheca Pedrosoi Fonseca and Leao, C. R., Soc. Biol. **89**: 762, 763, 1923.

Hormodendrum algeriensis Montpellier and Catanei, Ann. Dermat. et Syphil. **8**: 626–635, 1927.

Trichosporium Pedrosianum Ota, Jap. Jour. Dermat. and Urol. **28:** (4), 16–23, 1928.
Trichosporium Pedrosoi Langeron, Ann. Parasitol. **7:** 145–150, 1929.
Gomphinaria Pedrosoi Dodge, Med. Mycol, page 850, 1935.
Botrytoides Pedrosoi Moore and Almeida, Science **83:** 603, 1936.
Phialoconidiophora Guggenheimia Moore and Almeida, Science **83:** 603, 1936.

Plate 1, figures 1 to 4; plate 2, figure 2; plate 3, figures 7 to 10.

This fungus was first described by Brumpt in 1922 after a visit to South America in 1921. Several cases were immediately reported from South America and at the present time cases in North America (7), Guatemala (14), Algeria (15), and Puerto Rico (16) have yielded this fungus.

Of the seventeen strains of fungi studied, thirteen were found to be *Hormodendrum Pedrosoi.* In culture the gross characters of these fungi differed considerably. The several different macroscopic pictures of their growth on Sabouraud's glucose agar would seem to indicate that several species existed. In this respect two strains from Puerto Rico, one from South America, and one from Algeria were grown on Sabouraud's glucose agar (plate 1, figs. 1, 2, 4) (plate 2, fig. 2). The four were entirely different macroscopically in figuration, color, and size. On corn meal agar, however, these fungi produced little aerial mycelium, the growth being almost entirely within the agar (plate 1, fig. 3).

Although these strains of *Hormodendrum Pedrosoi* differed greatly in culture, they were identical microscopically. Conidiophores varying in length and number of cells bearing conidia in branching chain formation were developed in dendroid fashion from the aerial mycelium (plate 3, fig. 9). The conidia developed as buds on the tip of the conidiophore and in turn produced buds giving the branched chain condition of sporulation. In this type of conidial formation the youngest spore is at the distal end of the chain. Conidia were smooth-walled 2.5 μ x 7–13 μ next to the conidiophore and 1.5–2.5 μ x 2.5–5 μ at the end of the chain. This *Hormodendrum* type of conidial formation is the most prominent form to be seen in these cultures. Another type of conidiophore resembled that of the genus *Acrotheca* (plate 3, figs. 8, 10).

These conidiophores developed as terminal cells on aerial hyphae and as single lateral branches. At first the conidia were developed at the tip of the conidiophore but instead of developing chains as in the *Hormodendrum* type, other conidia were produced on the swollen tip in acropleurogenous fashion. As the swollen portion extended towards the base of the conidiophore other spores were borne on short protuberances. The conidia on these knotted club-shaped structures then budded to form chains exactly as seen in the *Hormodendrum* type (plate 3, fig. 9). This further development would exclude the genus *Acrotheca*. Other abortive types of *Acrotheca* conidiophores were produced laterally and sessilely on the aerial mycelium (plate 3, fig. 8). A third type of conidiophore, the *Phialophora* type, was found in eight of the thirteen strains studied. These were phialides produced laterally and terminally on the aerial mycelium of corn meal cultures (plate 3, fig. 7). They seem identical in size with those developed in cultures of *Phialophora verrucosa*. The egg-shaped conidia, however, were somewhat smaller 1.5–2 μ x 2–2.5 μ. The importance of these spores has yet to be determined. Although they were similar to the type of spores found in cultures of *Phialophora verrucosa*, they differed in two respects; they were smaller and we have not seen these spores germinate. Emmons and Carrion (21, 22) consider this type of sporulation as evidence of a phylogenetic relationship between *Phialophora* and *Hormodendrum*. Moore and Almeida (6) consider the development of these spores in much the same light. These spores, however, do not seem to be a part of the asexual development, conidial formation, of the cultures of *Hormodendrum* and in the light of recent work (17, 18) we would rather think of them as spermatia.

Hormodendrum Pedrosoi differs from *Hormodendrum compactum* by the loose arrangement of its conidial chains, the ovoid conidia which are easily dissociated, and the frequent occurrence of the *Acrotheca* type of conidiophore. *H. Langeroni* is easily differentiated by its round rough conidia and chains separated by well defined plugs (disjunctors). Compare plate 3, figs. 3, 9, 13.

Cultures studied:

Trichosporium Pedrosoi	38	South America
Trichosporium Pedrosoi	229	South America
Acrotheca Pedrosoi	268*	South America
Hormodendrum Pedrosoi	274	South America
Hormodendrum Pedrosoi	279*	South America
Hormodendrum Pedrosoi	280	South America
Hormodendrum Pedrosoi	69*	Puerto Rico
Hormodendrum Pedrosoi	267*	Puerto Rico
Hormodendrum Pedrosoi	275*	Puerto Rico
Hormodendrum Pedrosoi	284*	Puerto Rico
Hormodendrum Pedrosoi	269*	North Carolina
Hormodendrum Pedrosoi	297*	Guatemala
Hormodendrum algeriensis	281	Algeria (C.B.S.)

3. *Hormodendrum compactum* Carrión, Puerto Rico Journ. Pub. Health and Trop. Med. **10**: 543–545, 1935. Plate 2, figure 1; plate 3, figures 1 to 6.

This fungus was isolated from the hand and forearm of a farmer in Puerto Rico. The lesion has been considered a new clinical type by Carrion (19).

On Sabouraud's glucose agar (plate 2, fig. 1) the fungus resembled the North American strain of *Phialophora verrucosa*. It was slow growing and tended to become heaped and brittle. The surface was covered with a short nap-like aerial growth and was olive black.

Microscopically the aerial mycelium was poorly differentiated into terminal and lateral conidiophores at the tips of which short stubby buds developed and gave rise to long branching chains of subspherical conidia. The whole spore head was a compact mass not easily dissociated (plate 3, figs. 1, 2, 3). The subspherical conidia were smooth, olivaceous, 2.5–3 μ x 3.5–5 μ at the conidiophore, and 1.5–2 μ x 2–3 μ at the distal end of the chain (plate 3, fig. 4). The vegetative submatrical mycelium was 2.5–5 μ in diameter giving the appearance of swollen, torulose hyphae because of its wavy outline. This mycelium contained fat droplets and protoplasmic material (plate 3, fig. 6). *Phialophora*-like

* Those strains in which the *Phialophora* type of conidiophore was found when grown on corn meal agar.

phialides were also produced in this species when grown on corn meal agar. One or more may be produced on the terminal element of the mycelium (plate 3, fig. 5). The characteristic flask-shape phialides terminated by a cup-like formation were not unlike those phialides found in *Phialophora* cultures. They were 3.5 x 4.5 μ and the egg shaped conidia, 1.5 x 2 μ, were smaller than those of *Phialophora*. In this species as in *Hormodendrum Pedrosoi* these spores may be regarded as spermatia.

Hormodendrum compactum differs from the other species by the compact arrangement of its conidial chains which were not easily dissociated, by its subspherical conidia, and wide torulose submatrical mycelium. Compare plate 2, figs. 3, 9, 13.

Cultures studied:

Hormodendrum compactum 277.

4. *Hormodendrum Langeroni* Fonseca, Leão, and Nogueira-Penido, Sciencia Med. **5**: 563–573, 1927. Plate 2, figure 6: plate 3, figures 11 to 15.

This fungus was isolated first from a Brazilian showing several ulcerating lesions along the course of the lymphatics of the right hand and forearm (9). Clinically, however, the lesions suggested sporotrichosis. In 1934 Rotter and Chavarria (20) isolated this fungus from the right hand and forearm of a 70 year old farmer but the lesion in this case was typically that of chromoblastomycosis.

This species grew faster than the others obtaining a diameter of 55 mm. in 17 days on Sabouraud's glucose agar (plate 2, fig. 6). It produced a close powdery growth with several deep radial furrows and was dark green.

Microscopically on Sabouraud's glucose agar the hyphae appeared as olivaceous smooth thick walled cells with conidiophores developed at right angles to the aerial mycelium (plate 4, figs. 11, 15). Occasionally the smooth walled appearance was lost in old cultures and the hyphae had a crusted appearance. This was more noticeable in corn meal agar cultures (plate 3, fig. 13). The conidiophores developed perpendicularly on the aerial mycelium and were composed of cylindric (plate 3, fig. 15) or of long ovoid cells (plate 3, fig. 11). These ovoid cells were

similar to the branching spore heads of *H. Pedrosoi* (plate 3, fig. 9). They were joined by thick (1 μ) disjunctors and the whole element was easily dissociated. On Sabouraud's agar the terminal conidia were ovoid 3–4.5 μ x 5–7 μ, the intermediate cells making up the conidiophore were 3–4.5 μ x 6–11 μ. On corn meal agar long chains of round rough walled conidia with definite plug-like disjunctors were developed (plate 3, fig. 13). The youngest conidia at the tip were 2.5–3 μ in diameter, those nearest the ovoid cells from which they budded were 3.5–5 μ in diameter. Each of the dissociated elements carried disjunctors (plate 3, figs. 14, 12).

This species differed from all the others by its chains of round, rough walled easily dissociated conidia on corn meal agar, and the elementary type of conidiophore on Sabouraud's glucose agar. Compare plate 3, figures 3, 9, 13.

Cultures studied:

Hormodendrum Langeroni 282, C.B.S.

SEROLOGIC STUDIES—COMPLEMENT FIXATION

It has been shown previously that the serum of a patient infected with *H. Pedrosoi* had complement fixing antibodies for 13 of 14 strains of *Hormodendrum* from different cases of chromoblastomycosis (7). It was also shown that the same serum had complement fixing antibodies for two strains of *Phialophora* at a time when the titer to *Hormodendrum* was high, but no fixation could be obtained with this fungus when the titer to *Hormodendrum* was low. To obtain the high titered serum necessary to compare antigenic structures rabbits were immunized with two strains of *Hormodendrum Pedrosoi* 269, 281, and one strain each of *Hormodendrum compactum* 277, *Phialophora verrucosa* 283, and *Hormodendrum Langeroni* 282.

Since intravenous injection of suspensions of these fungi frequently caused death from emboli, large amounts of a dense suspension were injected intracutaneously. The methods of the preparation and injection of the fungi are given elsewhere in the paper. The local reactions to the suspensions of live fungi were variable; in a number of areas thick cheesy pus containing

numerous fungus bodies could be expressed, in other areas only firm nodules were formed. All rabbits remained healthy throughout the immunization and four months after the last injection only a few small nodules could be found.

TABLE 1

SERUM OF RABBIT IMMUNIZED WITH	ANTIGEN	DILUTIONS OF SERUM				
		1:2	1:4	1:8	1:16	1:32
H. Pedrosoi 269 (North Carolina strain)	H. Pedrosoi (N.C.)	4+	4+	4+	4+	1+
	H. Pedrosoi (Algeria)	4+	4+	4+	2+	0
	H. compactum (Puerto Rico)	4+	4+	4+	3+	0
	P. verrucosa (Uruguay)	4+	4+	4+	1+	0
	H. Langeroni (Costa Rica)	2+	2+	0	0	0
H. Pedrosoi 281 (Algerian strain)	H. Pedrosoi (N.C.)	4+	4+	4+	3+	1+
	H. Pedrosoi (Algeria)	4+	4+	4+	4+	2+
	H. compactum (Puerto Rico)	4+	4+	4+	3+	0
	P. verrucosa (Uruguay)	4+	4+	3+	0	0
	H. Langeroni (Costa Rica)	0	0	0	0	0
H. compactum 277 (Puerto Rican strain)	H. Pedrosoi (N.C.)	4+	4+	4+	4+	4+
	H. Pedrosoi (Algeria)	4+	4+	4+	4+	4+
	H. compactum (Puerto Rico)	4+	4+	4+	4+	4+
	P. verrucosa (Uruguay)	4+	4+	4+	4+	2+
	H. Langeroni (Costa Rica)	0	0	0	0	0
P. verrucosa 283 (Uruguayan strain)	H. Pedrosoi (N.C.)	3+	2+	0	0	0
	H. Pedrosoi (Algeria)	3+	3+	0	0	0
	H. compactum (Puerto Rico)	0	0	0	0	0
	P. verrucosa (Uruguay)	4+	4+	3+	1+	0
	H. Langeroni (Costa Rica)	0	0	0	0	0
H. Langeroni 282 (Costa Rican strain) C.B.S.	H. Pedrosoi (N.C.)	0	0	0	0	0
	H. Pedrosoi (Algeria)	2+	0	0	0	0
	H. compactum (Puerto Rico)	0	0	0	0	0
	P. verrucosa (Uruguay)	2+	2+	0	0	0
	H. Langeroni (Costa Rica)	4+	4+	4+	4+	3+

Complement fixation reactions were carried out with the same technique as previously described (7). The results of these tests may be seen in table 1. Although no far reaching conclusions can be made it is interesting to note that certain relation-

ships between these fungi which were evident morphologically were also evident serologically. In this respect, two strains of *Hormodendrum Pedrosoi*, 269 and 281, were identical morphologically except for the absence of the *Phialophora* type of conidial formation in strain 281. Since all other structures were comparable, however, we considered these fungi to be of the same species. Serological studies added further support to this adoption of a single species since the sera of rabbits immunized with *Hormodendrum Pedrosoi* 269 and 281 not only had a high titer of complement fixing antibodies for their homologous antigens but also produced a high titer for each other. Another species, *Hormodendrum compactum* 277, although its conidial formation differed from that of *Hormodendrum Pedrosoi*, its antigenic properties were similar. In this respect, table 1 shows that although *Hormodendrum compactum* and *Hormodendrum Pedrosoi* differ structurally they had a high titer of complement fixing antibodies for each other. The sera produced by these three fungi, *Hormodendrum Pedrosoi* 269, 281 and *Hormodendrum compactum* 277 contained complement fixing antibodies for *Phialophora verrucosa* 283, but to a slightly less degree than with their homologous antigens. On the other hand, sera of rabbits immunized with *Phialophora verrucosa* 283 and *Hormodendrum Langeroni* 282 contained complement fixing antibodies which were present in high titer only for the homologous fungus.

It would seem, therefore, that those fungi having a *Hormodendrum* and *Phialophora* relationship demonstrable by morphologic similarities also have a serologic relationship which can be demonstrated by the presence of similar antigenic properties.

DISCUSSION

It must be remembered that the genera of the fungi Imperfecti as now known present a hodge-podge of miscellaneous forms. This has been a natural result of changing methods of study and the inability to make descriptions of fungi in pure culture fit the older meager descriptions which were made from growth on natural substrates such as leaves, twigs, bark, etc. Many of the Imperfect genera, to be more clearly understood, need mono-

graphic study which would enlarge our concept of these genera. Such studies would necessitate the comparison of several fungi in culture and a rearrangement of clearly described forms. Because of the lack of such a study several fungi, have been shifted to various genera on the examination and interpretation of a single form. *Hormodendrum Pedrosoi* Brumpt 1922, a notable example of such shifting, has been placed in five different genera. Originally Brumpt (1) considered his fungus a *Hormodendrum* because the description of this genus seemed adequate; vegetative mycelium repent; conidiophores erect, septate, fuscous, branched; catenulate conidia acrogenous on the branches; conidia spherical to ovoid, unicellular, olive to brown (plate 3, figs. 9, 13).

Subsequent morphologic studies instead of proposing new genera might have enlarged this description by adding to it newly found structures. Thus, Terra, et al. (21) in 1922 after a superficial examination of the sporulating methods of this fungus proposed that it be placed in the genus *Acrotheca*. The following year Fonseca and Leão (a) found the fungus to be identical with Brumpt's and changed the name to *Acrotheca Pedrosoi*. In this genus, however, the conidia are borne singly on short sterigmata in acropleurogenous fashion from the swollen tip of conidiophores. Although in cultures of *Hormodendrum Pedrosoi* structures are found which correspond to this description (plate 3, figs. 8, 10), the conidia proliferate to form chains identical to those of *Hormodendrum* (plate 3, fig. 9). This catenulate method of spore bearing would exclude this fungus from *Acrotheca*.

In a further study Langeron (4) discarded *Acrotheca* and proposed the genus *Trichosporium* because he did not notice the sterigmata on which the spores were borne from the swollen conidiophore tip. His pictures, however, show catenulate spore formation from these tips which is inconsistent with the genus *Trichosporium*. He objected to *Hormodendrum* because his moniliform vegetative hyphae did not reveal the disjunctors between the cells. It is evident from his pictures that he mistook the vegetative moniliform mycelium for the spore chains which are borne acrogenously on the aerial hyphae in cultures of *Hormodendrum*. Both of these genera, therefore, must be replaced by

the old genus *Hormodendrum* as originally proposed. A third genus *Gomphinaria*, has been proposed (5) but this genus has already been reduced to synonymy with *Acrotheca* (22).

In their recent studies of the morphology of *Hormodendrum Pedrosi* Emmons and Carrion (23, 24, 25) were the first to show the development of *Phialophora* flask-shaped conidiophores in this fungus. Not only were *Phialophora* conidiophores and conidia found in all of the strains of *Hormodendrum Pedrosoi* examined by these investigators but they were also found in a saprophytic strain of *Hormodendrum*. As these structures were rare and scattered in the cultures and the *Hormodendrum* type predominated, the original name was retained and the newly found structures added to its description.

In contrast, a comparative study of these fungi by Moore and Almeida (6) has added two new genera to the already imposing list. The old genera *Acrotheca* and *Trichosporium* which have been shown above to be misinterpreted by those investigators proposing them have likewise been excluded in this recent study. Instead of returning those fungi called *Acrotheca* or *Trichosporium* to *Hormodendrum*, Moore and Almeida, after careful examination, found structures related to the genus *Botrytis*. Because the fungi were darkly colored, they could not be placed in the Mucedinaceae with *Botrytis* but had to be placed in the Dematiaceae in a new genus *Botrytoides*. Up to this time careful studies had shown that this fungus also could be placed in *Acrotheca* and *Trichosporium*. The new genus *Botrytoides*, however, was proposed in spite of confusion already existing. Not only was *Botrytoides* proposed but a new genus and species *Phialoconidiophora Guggenheimia* was also proposed for a new complicated organism showing *Hormodendrum*, *Acrotheca*, *Botrytoides* and *Phialophora*-like methods of sporulation. While Emmon's eleven and our eight strains showed this same phenomenon, they could still be retained as *Hormodendrum Pedrosoi* by adding the *Phialophora* structures to its description. The one strain examined by Moore and Almeida and regarded as a new genus and new species added nothing but another name to the already long list of synonymy. It is this type of investigation which adds to the confused status of classification of the fungi pathogenic for man.

If these newly found structures are to be regarded as spermatia, as we believe they should, it would be wiser to wait and describe a new genus and species only if the ascomycete resulting from a crossing or fertilization of the various strains has not previously been described.

Serologic studies (7) on the serum of a patient infected with *Hormodendrum Pedrosoi* demonstrated that complement fixing antibodies were present for 13 strains of *Hormodendrum* regardless of the geographical distribution of the cases from which the fungi had been isolated. Using sera of rabbits immunized with five strains of fungi from cases of chromoblastomycosis it was possible to demonstrate serologic relationships which were in agreement with the morphological studies. Thus, two strains of *Hormodendrum Pedrosoi*, 269 from North Carolina and 281 from Algeria, were identical morphologically except for the absence of *Phialophora* conidiophores and conidia in strain 281. All other structures, however, were identical and one species was indicated. The sera of rabbits immunized with strains 269 and 281 showed a high titer of complement fixing antibodies for their homologous antigen as well as for each other. This serves to confirm their identity. *Hormodendrum compactum* 277, on the other hand, was identical antigenically to strains 269 and 281, but its morphology was strikingly different. In this case, it might be said that *Hormodendrum compactum* is a different but very closely related species.

Phialophora verrucosa 283 differed markedly in one respect from *Hormodendrum Pedrosoi* and *Hormodendrum compactum* by the production of only the *Phialophora* type of conidia but was similar in another respect in that occasional *Phialophora* types were found in *Hormodendrum Pedrosoi* and *Hormodendrum compactum*. This type of remote relationship is suggested in the serologic studies since fixation occurred in the sera of rabbits immunized with *Hormodendrum Pedrosoi* 269 and 281 and *Hormodendrum compactum* 277 when *Phialophora verrucosa* was used as the antigen. *Hormodendrum Langeroni* 282 differed morphologically from the other fungi not only by the lack of the *Phialophora* type of sporulation but also by the extreme differ-

ences in the type of *Hormodendrum* conidiophores and conidia. This extreme difference was also demonstrated serologically by the lack of fixation when *Hormodendrum Langeroni* was tested with the sera of animals immunized against *Hormodendrum Pedrosoi* and *Hormodendrum compactum.*

CONCLUSION

Seventeen strains of fungi from cases of chromoblastomycosis of wide geographical distribution have been studied comparatively and it was found that thirteen were *Hormodendrum Pedrosoi* Brumpt 1922, two were *Phialophora verrucosa* Thaxter 1915, and there was one each of *Hormodendrum Langeroni* Fonseca, Leão, and Nogueira-Penido 1927 and *Hormodendrum compactum* Carrión 1935.

Three of these four fungi *Phialophora verrucosa*, *Hormodendrum Langeroni*, and *Hormodendrum compactum* were distinct morphologic entities and could be easily recognized by their distinctive conidiophores.

Hormodendrum Pedrosoi, however, showed a variety of conidial structures which have made the classification of this species difficult. When carefully examined, the *Hormodendrum* type of conidia formation was seen to predominate and all other proposed genera could be placed in synonymy. One structure, the *Phialophora*-type, suggests the development of spermatia in these fungi.

Serologic studies based on the sera of rabbits immunized with two typical strains of *Hormodendrum Pedrosoi*, 269 and 281, and one strain each of *Hormodendrum compactum* 277, *Hormodendrum Langeroni* 282, and *Phialophora verrucosa* 283 showed that complement fixing antibodies were present for the homologous antigen of each fungus.

The sera of rabbits immunized with *Hormodendrum Pedrosoi* 269, 281 and *Hormodendrum compactum* 277 not only had a high titer of complement fixing antibodies for their respective antigens but also had a high titer of complement fixing antibodies for each other.

The sera for *Phialophora verrucosa* 283 and *Hormodendrum Langeroni* 282 contained complement fixing antibodies which

were present in appreciable degree only for the homologous fungus.

A recent work by Moore and Almeida (Ann. Missouri Bot. Gard., **23**, 543–552, 1936) published while this paper was in press gave accurate descriptions of the new genera and species proposed for those fungi causing chromoblastomycosis. In view of this paper, rather than the earlier publication in Science (l. c.), the synonomy of *Hormodendrum Pedrosoi*, Brumpt, 1922, to be accurate, must be changed to the following:

Hormodendrum Pedrosoi Brumpt, Precis de Parasitol., ed. 3, 1105, 1922.
Acrotheca Pedrosoi Fonseca and Leao, C. R. Soc. Biol., **89**, 762, 763, 1923.
Hormodendrum algeriensis Montpellier and Catanei, Ann. Dermat. et Syphil., **8**, 626–635, 1927.
Trichosporium Pedrosianum Ota, Jap. Jour. Dermat. and Urol., **28**, 16–23, 1928.
Trichosporium Pedrosoi Langeron, Ann. Parasitol. Hum. et Comp., **7**, 145–150, 1929.
Gomphinaria Pedrosoi Dodge, Med. Mycol., 850, 1935.
Botrytoides monophora Moore and Almeida, Ann. Miss. Bot. Gard., **23**, 543–552, 1936.
Hormodendroides Pedrosoi Moore and Almeida, Ann. Miss. Bot. Gard., **23**, 543–552, 1936.
Phialoconidiophora Guggenheimia Moore and Almeida, Ann. Miss. Bot. Gard., **23**, 543–552, 1936.

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PLATES

PLATE 1

FIG. 1. Culture of *Hormodendrum Pedrosoi* 267 on Sabouraud's glucose agar; 4-week growth, actual size.

FIG. 2. Culture of *Hormodendrum Pedrosoi* 268 on Sabouraud's glucose agar; 4-week growth, actual size.

FIG. 3. Culture of *Hormodendrum Pedrosoi* 268 on corn meal agar; 4-week growth, actual size.

FIG. 4. Culture of *Hormodendrum Pedrosoi* 69 on Sabouraud's glucose agar; 4-week growth, actual size.


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PLATE 2

FIG. 1. Culture of *Hormodendrum compactum* 277 on Sabouraud's glucose agar; 17-day growth, actual size.

FIG. 2. Culture of *Hormodendrum Pedrosoi* 281 on Sabouraud's glucose agar; 17-day growth, actual size.

FIG. 3. Culture of *Phialophora verrucosa* 204 on Sabouraud's glucose agar; 17-day growth, actual size.

FIG. 4. Culture of *Phialophora verrucosa* 283 on Sabouraud's glucose agar; 17-day growth, actual size.

FIG. 5. Culture of *Phialophora verrucosa* 204 on Sabouraud's glucose agar; 17-day growth, actual size.

FIG. 6. Culture of *Hormodendrum Langeroni* 282 on Sabouraud's glucose agar; 17-day growth, actual size.


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PLATE 3

All figures are camera lucida drawings at a magnification of 2000X. Present reduction approximately 500×.

1–6. *Hormodendrum compactum* Carrión.

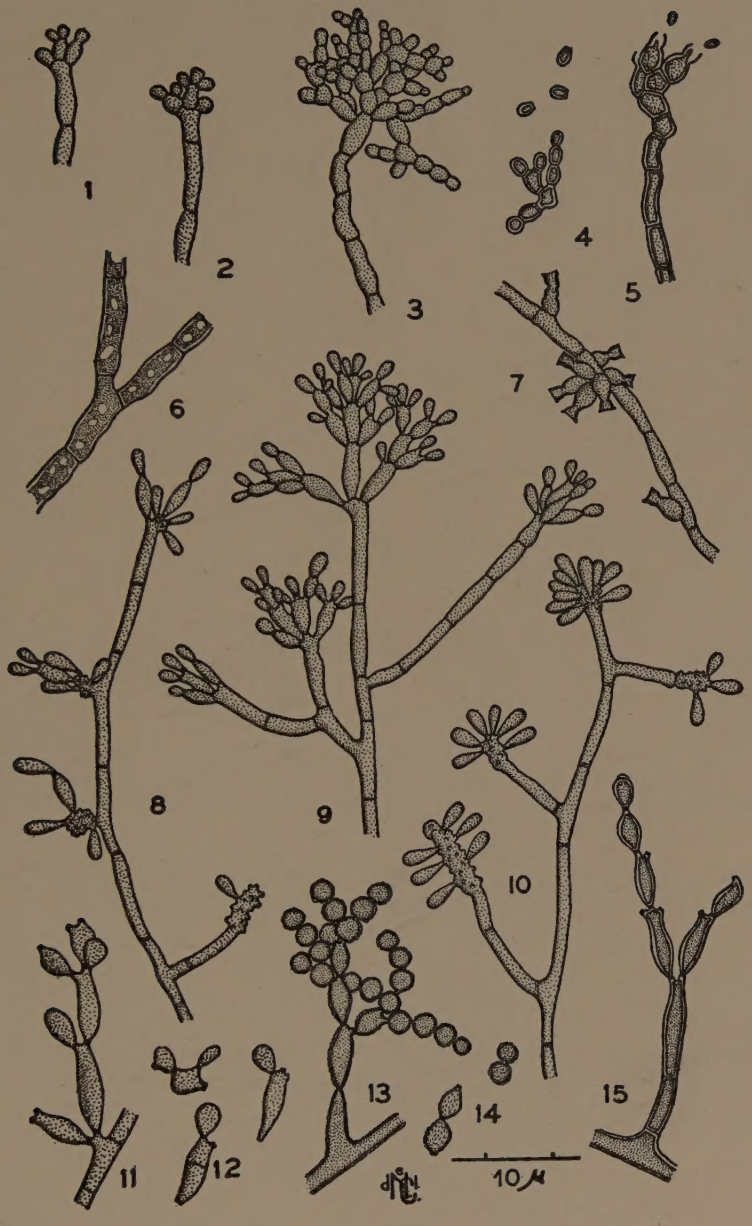
Fig. 1 and 2. Conidiophores in first stages of development.
Fig. 3. Mature conidiophore with branched conidial chains.
Fig. 4. Optical view of conidial chain.
Fig. 5. Thick walled hypha bearing *Phialophora*-type of conidiophore.
Fig. 6. Thick walled torulose hypha found in the agar.

7–10. *Hormodendrum Pedrosoi* Brumpt.

Fig. 7. *Phialophora*-type of conidiophores borne singly and in groups.
Fig. 8. Abortive *Acrotheca*-type of conidiophores; conidia are catenulate.
Fig. 9. Conidiophores of the *Hormodendrum*-type. The most prominent found in this species.
Fig. 10. *Acrotheca*-type of conidiophore with acropleurogenous development of the conidia.

11–15. *Hormodendrum Langeroni* Fonseca, Leão, and Nogueira-Penido.

Fig. 11 and 15. Conidiophores developed on Sabouraud's glucose agar.
Fig. 12. Conidia from culture on Sabouraud's glucose agar.
Fig. 13. Mature conidiophore and conidia developed on corn meal agar.
Fig. 14. Conidia from culture on corn meal agar.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